EVAPORATIVE LIGHT SCATTERING DETECTION IN QUANTITATIVE HPLC OF PAC MIXTURES AND COAL-TAR PITCHES

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Keywords: evaporative light scattering detector, HPLC, polycyclic aromatic compounds

INTRODUCTION

The term Polycyclic Aromatic Compounds (PACs) include a wide variety of classes of compounds. In turn, the number of possible PACs for each class is of astronomical proportion (1, 2). Environmental and fossil-fuel samples are composed of very complex mixtures of unknown PACs. The strategy for their analysis depends, among others, on the nature of matrix and PAC concentration, and involves cleanup/prefractionation steps and HPLC analysis. Therefore, HPLC detectors must be calibrated with pure reference standards for every substance to be quantified. However, only a relatively small fraction of PACs can unambiguously be identified and are commercially available (2). An ideal detector for chromatography of complex mixtures should provide uniform response factors for each separated compound or class of compounds. None of the conventional HPLC detectors (UV, Refractive Index, fluorescence) meet this requirement, neither for mixtures of unknown but well-separated pure peaks nor for compound-class fractionation of fossil fuels (where other additional problems can occur, such as presence of very heavy and polar PACs, quenching problems using fluorescence detection, need of tedious absolute calibrations, etc.)

It has been reported that the Evaporative Light Scattering Detector (ELSD) enables all types of non-volatile solutes to be detected (3, 4), although it has recently been reported that solutes having a lower volatility than the mobile phase can be analyzed working at mild temperatures (5). Detector response has been reported to be quite independent of the chemical composition of the solute. However, very different response factors were reported in the past in the case of semi-volatile PACs (6). This work intends: i) to evaluate the possibility of the application of ELSD in order to quantify PACs in complex mixtures, ii) to theoretically justify the responses of the studied PACs, and iii) to lay the groundwork for application to fossil-fuel characterization.

EXPERIMENTAL

The HPLC system consisted of: a Waters 510 pump, a 7725i Rheodyne injector, a PL-Gel Mixed-E (30 cm length, 3 μm particle size with mixed pore distribution), and a PL-EMD 950 ELSD. GPC-grade-Tetrahydrofuran (THF) stabilized with BHT was used as mobile phase. Flow rate was 1 ml/min. The output signal from the detector was fed into a Fluke Hydra 2620 multichannel data acquisition unit, and stored in an HP 100LX computer for data acquisition. Raw data were further reprocessed using an adequate spreadsheet in a 486 PC clone.

The operating mode of ELSD is as follows: the effluent from the chromatographic column enters a Venturi nebulizer and is converted into an aerosol by a stream of air (12 l min^{-1}). Venturi consists of a stainless steel capillary tube (0.25 mm i.d., 1.59 mm o.d.) carrying the column effluent, which is surrounded by a larger tube (2 mm i.d.). The fine droplets formed are then carried out through a temperature controlled drift tube which causes evaporation of the mobile phase to form small particles of non-volatile solute. This cloud of solute passes through a white light in the broad-band 400-700 nm, which produces light scattering, which in turn is detected by a photomultiplier at a 60° angle. The evaporation temperature used was 30°C.

The amounts (μg) of the studied PACs reported throughout the text correspond to the mass effectively injected. The response factor of each standard is defined as its corresponding area, A (counts as μV s^{-1}) per mass unit, m (μg). Only absolute response factors are used throughout this paper.

RESULTS AND DISCUSSION

Quantification of PACs using ELSD

In a first step of the research, detector response (A) vs sample load (m) was evaluated for pure PACs, and binary mixtures of PACs. In each mixture injected, either coronene or 2-hydroxycarbazole was used as reference standard due to their adequate retention time using the above-mentioned column. Although adequate regression coefficients ($r = 0.999$) were obtained for the studied PACs using linear regressions, deviations from the theoretical linearity at low sample loads were evidenced by: i) significantly lower response factors for each pure PAC as sample load decreased (Table 1), ii) in the case of mixtures, greater differences between area percentages from the chromatogram and known mass percentages (hereafter referred as to A-m differences) at low sample loads (Table 1), and iii) a better fit of experimental data to logarithmic regressions ($\log A = \log a + b * \log m$, from $A = a * m^b$). Parameters from both types of regression for some different PACs are given in Table 2. Values of a and b are similar for the studied PACs despite their different chemical nature, suggesting similar responses. The physical meaning of b, and the agreement between the experimentally obtained and

theoretically calculated *b* values for the studied PACs are explained later in this work, as well as a theoretical justification of linearity deviation. This is inherent to ELSD regardless of the volatility/involatility of the PACs. Recent results reported by Dreux el al. (5) showed a similar behavior in the case of glucose analysis on an octadecyl bonded silica when using either ELSD or Refractive Index detector. They demonstrated that linearity deviation is even larger in the latter case. The sample load range in which ELSD behaves in a non-linear fashion depends on the considered PAC (Table 1, results at 20 and 40 μg). Results from Table 1 at 40 μg (in the linear zone of ELSD) show that the studied 3-6 ringed, semi-volatile polycyclic aromatic hydrocarbons (PAHs) can be analyzed using ELSD under the described mild conditions with A-m differences lower than 6 % w/w. The same can be said for other analyzed hydroxy-PAHs, and heteronuclear PACs. All these standards presented quite uniform response factors despite their different chemical natures. Therefore, ELSD can be used in this zone *for the analysis of complex mixtures of PACs in which there are unknown components, avoiding any calibration step*. This is an advantage with regard to the other conventional HPLC detectors, such as UV, Refractive Index and fluorescence. At low sample loads, ELSD needs a calibration step and can be useful for mixtures of known PACs, in the same manner than the other HPLC detectors. From the point of view of quantitative accuracy, *b* does not have to be equal or close to unity providing an accurate value is known (7).

Are low-sized PACs really underestimated using ELSD?

The chromatographic quantification of PACs and other related compounds (with b.p. and MW lower than 285°C and 300 u.m.a., respectively) using ELSD was questioned in the past (6, 8). However, our results show reasonably uniform response factors in the linear zone of ELSD in the case of PACs with these characteristics. A partial volatilization of all the studied PACs due to the nebulization and evaporation must be discarded, given the negligeable volatility, for instance, of coronene (Po = 1.63 * 10^{-3} mm Hg at 150°C) at the mild conditions used (30°C). Semi-volatile PAHs with 3-6 aromatic rings, small-sized N-heteronuclear PACs and hydroxy-PAHs present similar response factors to hardly volatile coronene, and therefore can be analyzed using mild working conditions.

The impossibility of analyzing PACs using ELSD was exclusively attributed to the supposed volatility of these compounds. Although this should not be discarded in some cases, results presented here suggest that the values of the corresponding response factors reported in the literature were estimated at low loads and/or higher temperatures than those used here. As well, there have been changes in the configuration of nebulizers in the newer models with respect to the older ones. Nebulization directly influences particle diameter, which in turn affects the sensitivity of ELSD for different solutes, as explained in the last section of this work.

It must be stressed that the volatility of a given compound cannot merely be predicted from its b.p. and MW (3). For instance, although acenaphthene and 2-naphthol have similar b.p. and MW, the former showed no signal from ELSD under the conditions used, while the latter gave a high response factor. Vapor pressure at a given temperature (if possible) is a better parameter to compare relative volatilities although it should be taken with caution because ELSD evaporates in an air flow.

However, there is a value of vapor pressure for a given temperature over which PACs cannot be analyzed using ELSD. Thus, two-ringed PACs (naphthalene, 2-methylnaphtalene), and low-sized, non-planar, hydrogenated PACs (acenaphthene, fluorene), showed either no signal or an important loss of response at 30°C, as expected given their high vapor pressures.

Agreement between theoretical and experimental data on evaporative light scattering response of PACs. Why are response factors of studied PACs reasonably uniform under the used working conditions?

The stages involved in evaporative light scattering detection are: nebulization of the HPLC effluent, evaporation of the mobile-phase droplet cloud, and scattering of incident light.

Nebulization depends on the mobile phase properties and flow rate, nebulizer geometry and nebulization conditions, through Mugele and Evans' droplet size distribution (9), and the equation of Nukiyama and Tanassawa, as reported by Van der Meeren et al. (10). Details of all calculations presented here are reported elsewhere (11). The droplet size distribution (in volume) under our working conditions is given in Figure 1.

After evaporating, the diameter of solute particles (d_s) can be related to the concentration, *C*, through

$$d_s = d_m (C / \rho_s)^{1/3}$$

where ρ_s is the solute density, and d is each value of the droplet diameter distribution after nebulization. *C* is function of both time and chromatographic conditions according to

$$C = \frac{X/10^9}{Q_l} \frac{\exp\left[-(t_r - t)^2 / (2sd^2)\right]}{(2\Pi)^{1/2} sd}$$

where X is the sample load, Q_f is the mobile phase flow rate and sd and t_r are the standard deviation and retention time of the chromatographic peak, respectively. This means that each point of a given chromatogram has its corresponding particle size distribution. The highest diameters in the distributions correspond to the maximum of each peak. Figure 2 shows nine size distributions corresponding to the first half of the chrysene peak. Figure 3 gives the particle size distributions at peak maxima in the cases of chrysene and coronene. Particle size distributions for the studied PACs are similar because their densities are not very different (between 1100 and 1300 kg m^3, approximately).

ELSD is a detector which presents different sensitivities with regard to the scattering domain of work (Rayleigh, Mie, reflexion-refraction). These domains are determined by the ratio of a characteristic dimension (e.g. diameter, d_e) of the solute particle to the wavelength(s) of the incident light beam (λ) (12). It is very important to work in similar d_e/λ ranges in order to have homogeneous response factors for the components of a complex mixture. Rayleigh scattering is predominant when d_e/λ is smaller than 0.1. In this domain, the intensity (I) of scattering increases very rapidly with increasing particle diameter (d_e^6). I is lower for particles in the Rayleigh domain than for other domains. Mie scattering becomes preponderant when d_e/λ is greater than 0.1. In this domain, I increases as the fourth power of diameter. For greater d_e/λ ratios (approximately 2), reflexion and refraction become dominant and sensitivity increases. (I is function of diameter squared). I from spherical particles can be calculated *for any size* using Mie's theory. Therefore, when I is expressed in function of the concentration, (13) through particle diameter ($d_e = d_m^* (C / \rho_s)^{1/3}$), then $I = f(C^2)$, $f(C^{1.33})$ or $f(C^{0.66})$, for Rayleigh, Mie or reflexion-refraction domains, respectively. In Table 2 it can be seen that experimentally obtained b values for PACs are between 1.29 and 1.47, that is, near to the theoretical value for Mie's domain. Calculations of d_e/λ from particle size distributions are in agreement with this. The highest d_e/λ for chrysene (at 40 µg, at the maximum of the peak, and for λ=400 nm) is 1.05. The homogeneous response of PACs is illustrated by the similar values of d_e/λ for chrysene and coronene (Figure 3).

Application of ELSD to HPLC-compound class fractionation and HPLC-GPC of fossil fuels

Besides the above-mentioned quantitative application of ELSD for analyzing mixtures of unknown PACs, this detector presents several advantages with respect to Refractive index detector, in the case of compound-class fractionation of fossil fuels, in general: possibility of using gradients of eluants and typical eluants of reversed phase, rapid stabilization with temperature, and adequate baseline (no negative peaks, no solvent fronting). More particularly, coal-tar pitches mainly consist of PAHs and their heteronuclear analogues (14). Therefore, the PACs studied in this work represent quite well a part of PACs found in coal-tar pitches. These are usually analyzed using HPLC-compound class characterization after a chromatographic prefractionation. According to our results, ELSD could be used to quantify this type of analysis. GPC-ELSD has been carried out to obtain comparative curves of size distribution for coal-tar pitches, using the same column as that used here (15). Given that no discrete peaks are obtained with this technique, more research is needed to understand the influence of nebulization and chromatographic conditions on particle diameter in order to perform semi or quantitative analyses.

ACKNOWLEDGEMENTS

The authors are grateful to Spanish DGICYT (Project PB93-0100) and ECSC (European Steel & Coal Community, Project 7220-EC/765) for their financial support

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Table 1. Response factors (A/m), and differences between area and mass percentages (Δ) of PAC standards in binary mixtures at several sample loads using either 2-hydroxycarbazole (HCBZ) or coronene (CRN) as references[1].

Standards	40 μg		20 μg		8 μg
	Δ[2]	A/m	Δ[2]	A/m	A/m
7,8-benzoquinoline	0.8	1.188	15.7	0.475	0.213
diphenyldisulfide	1.0	1.187‡	7.0	0.621‡	0.347
coronene	1.5	1.308	8.2	0.870	0.321
perylene	1.5	1.383	7.2	0.754	0.410
2-hydroxycarbazole	1.5	1.390‡	8.2	1.220‡	0.565
Benzo[a]pyrene	2.5	1.407	5.2	0.794	0.435
tianthrene	2.5	1.291	1.3	0.974	0.446
anthracene	2.6	1.002	7.0	0.733	0.536
acridine	2.6	1.523	4.7	1.138	0.319
phenoxazine	3.4	1.584‡	10.1	0.994‡	0.562
2-naphthol	4.2	1.301‡	3.4	0.782‡	0.243
9-phenanthrol	4.4	1.090‡	1.7	1.166‡	0.573
pyrene	4.7	1.478	5.1	1.147	0.563
fluoranthene	5.8	1.436	6.5	0.941	0.390
2,3-benzofluorene	6.1	0.850	8.3	0.638	0.320
9-hydroxyfluorene	6.7	1.454‡	5.8	1.083‡	0.550
carbazole	8.7	0.917‡	7.6	0.634‡	0.389
chrysene	9.7	0.857	9.2	0.647	0.345
phenanthrene	9.0	0.775	22.2	0.419	0.225

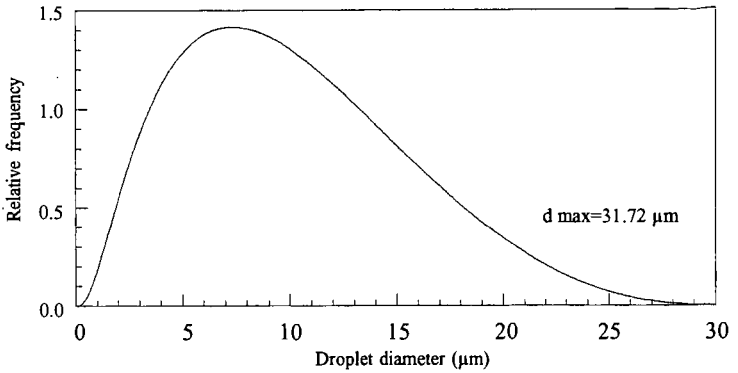
[2] Absolute value.[1] Superscript ‡ indicates that coronene was the reference; the remaining refer to HCBZ.

Table 2.- Fitting of ELSD response vs sample load for three studied PACs.

	2-hydroxycarbazole	coronene	chrysene
Linear regression			
slope	1.542	1.537	0.994
intercept	-6.887	-10.07	-3.66
r	0.9995	0.9923	0.999
error %[1] at 5 μg	17.0[2]	140.2	74.9
Logarithmic regression			
b	1.291	1.474	1.344
a	-0.340	-06677	-0.5897
r	0.9984	0.9999	0.9963
error %[1] at 5 μg	-2.9[2]	0.04	-10.5

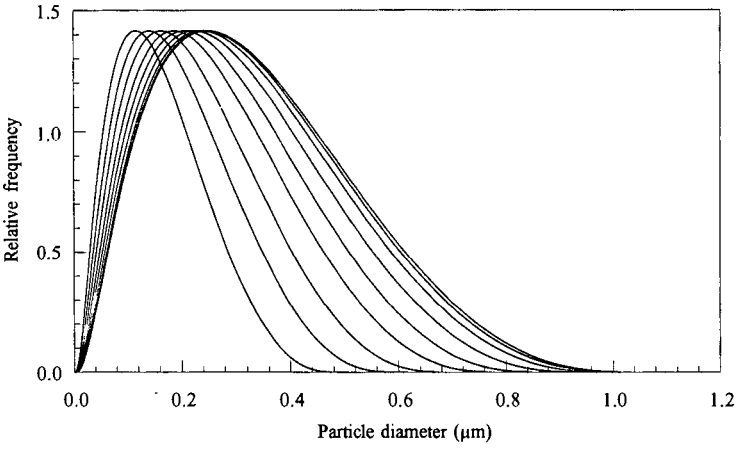
[1] Error % between the corresponding experimental value and that predicted from the corresponding fitting curve. [2] In these cases, error % at 8 μg.

Figure 1



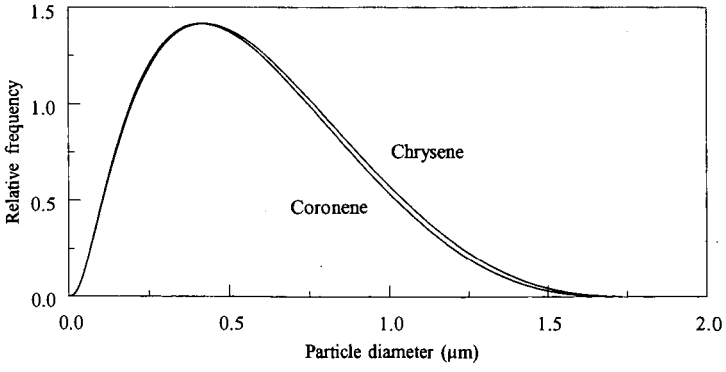
Droplet size distribution (in volume) from the nebulization of THF in a venturi.

Figure 2



Nine particle size distributions (in volume) corresponding to chrysene elution (5 µg load) from t=tr - w/2 (start of peak) to t=tr (peak maximum)

Figure 3



Particle size distributions at peak maxima (40 µg) for chrysene and coronene